

CLAIMS

1. A uracil-requiring gene-disrupted yeast
wherein URA3 gene of chromosomal DNA is disrupted by
5 the homologous recombination with a URA3 DNA fragment.
2. A histidine-requiring gene-disrupted yeast
wherein HIS5 gene of chromosomal DNA is disrupted by
the homologous recombination with an HIS5 DNA fragment.
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3. An adenine- and uracil-requiring gene-disrupted yeast
wherein ADE1 gene and URA3 gene of chromosomal DNA
are disrupted by the homologous recombination with an ADE1
15 DNA fragment and URA3 DNA fragment.
4. An adenine- and histidine-requiring gene-
disrupted yeast
wherein ADE1 gene and HIS5 gene of chromosomal DNA
20 are disrupted by the homologous recombination with an ADE1
DNA fragment and HIS5 DNA fragment.
5. A uracil- and histidine-requiring gene-disrupted yeast
wherein URA3 gene and HIS5 gene of chromosomal DNA
25 are disrupted by the homologous recombination with a URA3
DNA fragment and HIS5 DNA fragment.
6. An adenine-, uracil- and histidine-requiring
30 gene-disrupted yeast
wherein ADE1 gene, URA3 gene and HIS5 gene of
chromosomal DNA are disrupted by the homologous
recombination with an ADE1 DNA fragment, URA3 DNA fragment
and HIS5 DNA fragment.
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7. The gene-disrupted yeast according to any one of Claims 1 to 6,

wherein the yeast is one belonging to the genus Candida, the genus Clavispora, the genus Cryptococcus, the 5 genus Debaryomyces, the genus Lodderomyces, the genus Metschnikowia, the genus Pichia, the genus Rhodosporidium, the genus Rhodotorula, the genus Sporidiobolus, the genus Stephanoascus, or the genus Yarrowia.

10 8. The gene-disrupted yeast according to any one of Claims 1 to 6,

wherein the yeast belongs to the genus Candida.

9. The gene-disrupted yeast according to any one of 15 Claims 1 to 6,

wherein the yeast is the albicans species, ancudensis species, atmosphaerica species, azyma species, bertae species, blankii species, butyri species, conglobata species, dendronema species, ergastensis species, 20 fluviatilis species, friedrichii species, gropengiesseri species, haemulonii species, incommunis species, insectrum species, laureliae species, maltosa species, melibiosica species, membranifaciens species, mesenterica species, natalensis species, oregonensis species, palmioleophila 25 species, parapsilosis species, pseudointermedia species, quercitrusa species, rhagii species, rugosa species, saitoana species, sake species, schatavii species, sequanensis species, shehatae species, sorbophila species, tropicalis species, valdiviana species, or viswanathii 30 species of the genus Candida.

10. The gene-disrupted yeast according to any one of Claims 1 to 6,

wherein the yeast is Candida maltosa.

11. The URA3 gene-disrupted yeast according to Claim
1
which is Candida maltosa U-35 (FERM P-19435).
- 5 12. The HIS5 gene-disrupted yeast according to Claim
2
which is Candida maltosa CH-I (FERM P-19434).
- 10 13. The ADE1 gene- and URA3 gene-disrupted yeast
according to Claim 3
which is Candida maltosa UA-354 (FERM P-19436).
- 15 14. The ADE1 gene- and HIS5 gene-disrupted yeast
according to Claim 4
which is Candida maltosa AH-I5 (FERM P-19433).
- 20 15. The URA3 and HIS5 gene-disrupted yeast according
to Claim 5
which is Candida maltosa HU-591 (FERM P-19545).
- 25 16. The ADE1 gene-, URA3 gene- and HIS5 gene-
disrupted yeast according to Claim 6,
which is Candida maltosa AHU-71 (FERM BP-10205).
- 30 17. A transformant of the gene-disrupted yeast
according to any one of Claims 1 to 16,
which is transformed with a DNA sequence containing
an isogene or heterogene.
- 35 18. A process for producing a gene expression
product
which comprises harvesting an expression product of
an isogene or heterogene from a cultured product obtainable
by culturing the transformant according to Claim 17.

19. The process for producing a gene expression product according to Claim 18,
wherein the gene expression product is a polyester.

5 20. A yeast transformant
which is introduced with a polyhydroxyalkanoic acid synthase gene and an acetoacetyl CoA reductase gene, and both or either of these genes being introduced in 2 or more copies.

10 21. The yeast transformant according to Claim 20,
wherein a peroxisome-targeting signal is added to a polyhydroxyalkanoic acid synthase gene and an acetoacetyl CoA reductase gene.

15 22. The yeast transformant according to Claim 20 or 21,
wherein a promoter and terminator functioning in yeast are connected to a polyhydroxyalkanoic acid synthase 20 gene and acetoacetyl CoA reductase gene.

25 23. The yeast transformant according to any one of Claims 20 to 22,
wherein the yeast belongs to the genus Candida.

24. The yeast transformant according to any one of Claims 20 to 22,
wherein the yeast is the albicans species, ancudensis species, atmosphaerica species, azyma species, bertae species, blankii species, butyri species, conglobata species, dendronema species, ergastensis species, fluvialis species, friedrichii species, gropengiesseri species, haemulonii species, incommunis species, insectrum species, laureliae species, maltosa species, melibiosica species, membranifaciens species, mesenterica species,

natalensis species, oregonensis species, palmioleophila species, parapsilosis species, pseudointermedia species, quercitrusa species, rhagii species, rugosa species, saitoana species, sake species, schatavii species, 5 sequanensis species, shehatae species, sorbophila species, tropicalis species, valdiviana species, or viswanathii species of the genus Candida.

25. The yeast transformant according to any one of
10 Claims 20 to 22,
wherein the yeast is Candida maltosa.

26. The yeast transformant according to any one of
Claims 20 to 25,
15 wherein the polyhydroxyalkanoic acid synthase gene
codes for an enzyme or mutant derived from Aeromonas caviae
having the amino acid sequence shown under SEQ ID No:5.

27. The yeast transformant according to Claim 26,
20 wherein the polyhydroxyalkanoic acid synthase gene
derived from Aeromonas caviae codes for a
polyhydroxyalkanoic acid synthase mutant obtainable by
applying at least one of the following amino acid
substitutions from (a) to (h);
25 (a) substitution of Ser for Asn-149
(b) substitution of Gly for Asp-171
(c) substitution of Ser or Gln for Phe-246
(d) substitution of Ala for Tyr-318
(e) substitution of Ser, Ala or Val for Ile-320
30 (f) substitution of Val for Leu-350
(g) substitution of Thr, Ser or His for Phe-353
(h) substitution of Ile for Phe-518.

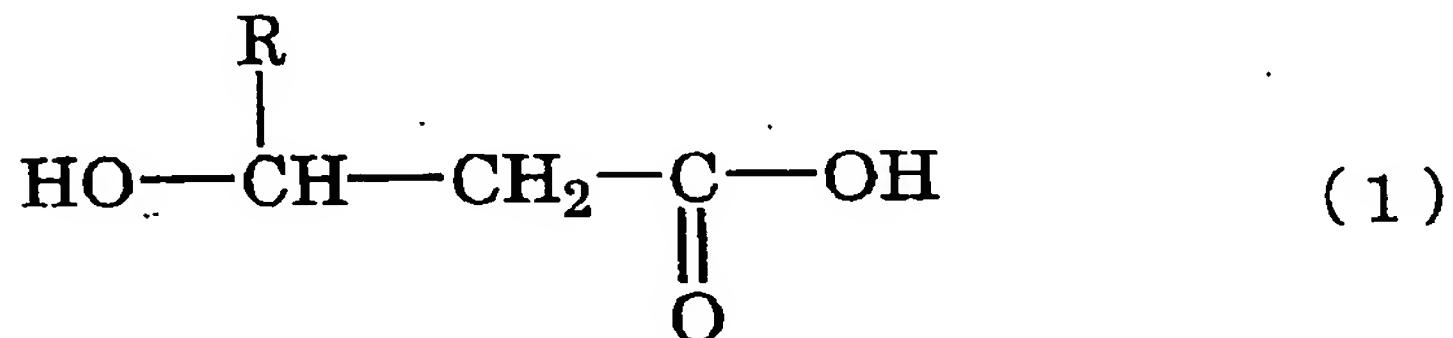
28. The yeast transformant according to any one of
35 Claims 20 to 27,

wherein the acetoacetyl CoA reductase gene codes for an enzyme or mutant derived from Ralstonia eutropha having the amino acid sequence shown under SEQ ID NO:6.

- 5 29. The yeast transformant according to any one of Claims 20 to 28,

 wherein the polyhydroxyalkanoic acid is a copolymer obtainable by copolymerizing 3-hydroxyalkanoic acid represented by the following general formula (1);

10 [Chemical 1]



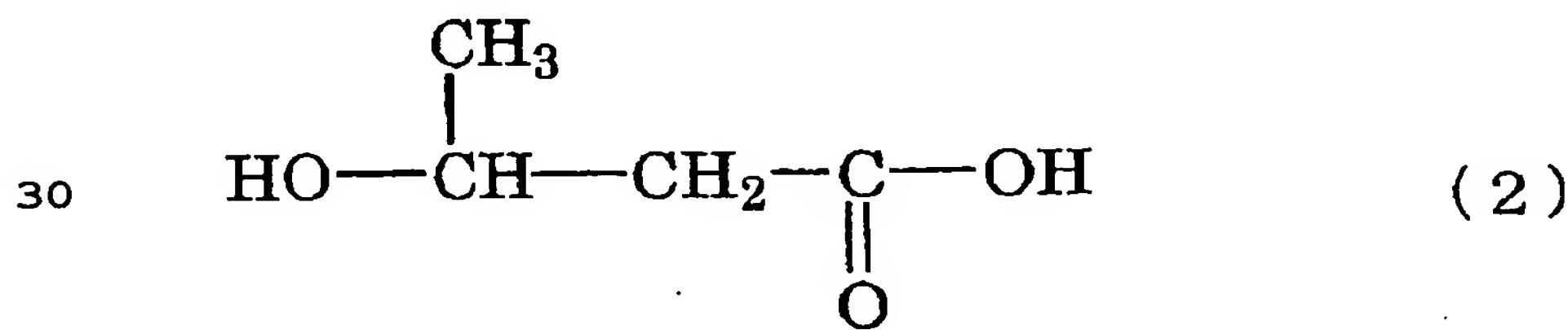
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 in the formula, R represents an alkyl group having 1 to 13 carbon atoms.

- 30 30. The yeast transformant according to any one of Claims 20 to 28,

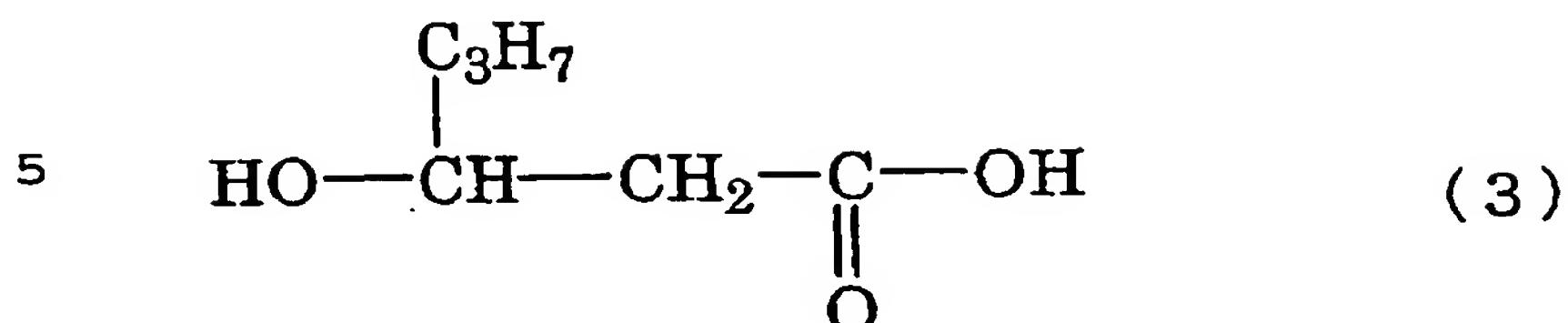
 wherein the polyhydroxyalkanoic acid is a copolyester obtainable by copolymerizing 3-hydroxybutyric acid represented by the following general formula (2) and 3-hydroxyhexanoic acid represented by the following general formula (3).

25 [Chemical 2]



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[Chemical 3]



31. A process for producing a polyester using the
10 yeast transformant according to any one of Claims 20 to 30,
which comprises harvesting a polyester from a
cultured product obtainable by culturing said yeast
transformant.

15 32. A method for controlling the molecular weight of
a polyester in producing a polyester using the yeast
transformant according to any one of Claims 20 to 30,
which comprises controlling the number of acetoacetyl
CoA reductase gene in the yeast transformant.

20 33. A method for controlling a hydroxyalkanoic acid
composition of a polyester in producing a polyester using
the yeast transformant according to any one of Claims 20 to
30,
which comprises controlling the number of a
polyhydroxyalkanoic acid synthase gene in the yeast
transformant.

25 34. A method for recovering a selective marker
which comprises carrying out the intramolecular
homologous recombination in Candida maltosa having ADE1
30 gene as a selective marker gene to remove said ADE1 gene.

35 35. The method for recovering a selective marker
according to Claim 34,

wherein a part of ADE1 gene is ligated to the upstream or downstream of ADE1 gene.

36. The method for recovering a selective marker
5 according to Claim 34 or 35,

wherein ADE1 gene has the base sequence shown under SEQ ID NO:7.

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